A CHEMICAL SIGN OF LIFE.[1]

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From the earliest time to the present, our criterion of life has been connected with the changes which are brought about by death. The locomotive power of living matter is one of the first things that disappears when it dies. Thus the idea of movement erroneously led the simple minds of our ancestors to believe that wind, fire, thunder and water had life. By close study of the locomotion of living matter, however, we have gradually traced this part of living phenomena to irritability of tissue—the property characteristic to living tissues only. This protoplasmic irritability is not only the potential of living matter, to give rise to physical changes when a stimulus is applied to it, but the fundamental and the only characteristic which is common to all the living as long as they possess the power to perform their own functions. And it is this property, according to Professor Mathews,[2] that not only is the most probable point of attack of natural selection but is also one of the main factors which determines phylogenetic development of organisms.

The presence of this irritable property in tissue—the universal sign of life—cannot easily be determined in all living tissues. The most common way of determining irritability is the physical changes brought about by a stimulus. This physical change, however, is not an unfailing indication of a response of the tissue against stimulation. Several tissues in the animal, such as nervous tissues, and an abundance of examples in the plant kingdom, do not manifest at all any visible change, when stimulated. Not only those living do not show such mechanical

[1] I wish to thank Dr. F. R. Lillie, through whose kindness I was occupying a table in the Marine Biological Laboratory, Woods Hole, Mass., at the time when the apparatus for these experiments was made.

[2] Mathews, *Amer. Naturalist*, XLVII., p. 94, 1913.

changes while functioning, but several non-living matters constantly undergo physical changes when disturbed by external conditions. Therefore it has been customary for the biologist to decide whether or not matter is living by several other accompanying changes during the performance of a function. Such changes have been measured by rise of temperature, production of CO_2, histological variation before and after the stimulation, and electrical response. None of these functional changes when taken individually can be considered as a specific sign of life.

Although Herzen claims that under certain conditions of local narcosis, the nerve fiber may give an action current although no muscular contraction follows, and O. B. Ellison recently demonstrated by the use of cinchonamine hydrochloride the absence of negative variation without abolishing excitability, yet according to Waller the presence of life can be demonstrated by an electrical change. In his admirable book on the "Signs of Life,"[1] he states that chemical change is a sign of life and an electrical change is a sign of a chemical change; therefore the electrical change is the sign of life. It is very interesting to note in the case of a dry seed, he could measure equally well quantitatively the different electrical changes according to the different ages which characterize the different degrees of vitality of the seed, in spite of the fact that he could not detect any chemical change which usually produces CO_2. He concludes, however, that it is possible, or rather certain that our method of chemical investigation is not refined enough to reveal to us the smallest and most infinitesimal change that may be going on in apparently dry or dormant seed. It was this conclusion of his that suggested to me the desire to make an inquiry to ascertain whether or not I could find some new method by which an easy sign of life may be observed.

When I had constructed a new apparatus,[2] which can detect CO_2 as small as 0.0000001 gram, the measurement of irritability became much simpler, for with it we established a few new facts which deal with the fundamental nature of protoplasmic irritability. The idea that irritability in general is closely associated

[1] Waller, "Signs of Life," New York.

[2] *Am. J. of Physiol.*, XXXII, p. 137, 1913.

with chemical phenomena has been set forth long ago. Professor Mathews, in his paper on "Animal Oxidation," expressed the idea that cellular respiration must be the fundamental process of all organic activity around which all functional phenomena are intimately connected. Later in his study on the action of ether and other anæsthesias on an anaerobic tissue he confirmed his idea that all the substances that affect irritability must necessarily attack the tissue respiration first. With the aid of the new apparatus, the direct evidence for his hypothesis has been brought forth in connection with the study of metabolism of the nerve fiber, in which three fundamentally interesting facts have been established. In the first place, the most excitable tissue of all the protoplasm, the nerve fiber, is constantly undergoing chemical changes, giving off CO_2. In the second place, when this tissue is stimulated CO_2 production is greatly accelerated, giving more than double the amount. Finally, the rate of CO_2 is greatly influenced by conditions such as anæsthesia, which are known to affect tissue irritability, showing a direct relation between respiration and excitability.[1]

Before I had concluded, from these facts, that all the irritable tissues must respire and should give off more CO_2 when stimulated, a crucial experiment was done on a dry seed. Waller's conclusion has been fully realized. A living dry seed not only gives off CO_2 quantitatively proportional to its weight, but also gives off more CO_2 when stimulated, a phenomenon true to living seeds only. These facts enable me to propose a new sign of life, namely, a chemical sign. The criterion is simple. If we are given a tissue which gives more CO_2 when stimulated, the tissue must be alive; it is excitable. A discovery of a remarkably simple method of stimulation made this chemical sign of life much more easily practicable to all living tissues. Simple mechanical crushing of the living tissue is the new method. I have already argued elsewhere[2] that the phenomenon that the nerve gives off more CO_2 when crushed, is due mainly to an extreme stimulation and that it is characteristic of living, excitable tissues only. Therefore without any attempt to settle the question as to how CO_2 production is increased, we can use

[1] *Am. J. of Physiol.*, XXXII., pp. 107–136, 1913.

[2] *Am. J. of Physiol.*, XXXII., p. 121, 1913.

this method as a means to denote protoplasmic irritability, as long as we can abolish this sign of life by rendering the tissue unexcitable. The nerve, dry seeds, including wheat, oats, rice and several other living tissues from animal and plant kingdoms, if treated with ether, or killed, can never be made to produce more CO_2 by crushing, whereas, a tissue however little CO_2 it may produce normally will give off more CO_2 when crushed, provided it is living. From these general findings, I conclude that this method can be used to detect vitality of protoplasm under normal conditions.

The details of this simple means of finding the sign of life are as follows:

The biometer is used. This is an apparatus which has two respiratory chambers, each furnished with a cup in which a drop of barium hydrate can be introduced. With it, the comparative outputs of CO_2 as small as 0.0000001 g. from the two tissues can be estimated simultaneously.[1] Two dry living kernels of the same seed, with equal weights, are chosen. One is placed in the right chamber, and the other is crushed and placed in the left. After the necessary cleaning of the apparatus with CO_2-free air, a drop of the barium hydrate is introduced upon each cup in both chambers. By watching the drop, it would become obvious that the crushed seed is giving off more CO_2 than the uninjured, as indicated by the speed of formation and quantity of precipitate of the barium carbonate. Not only such distinction between two can be observed, within a few seconds, in the case of the tissue, which normally gives off a comparatively large quantity of CO_2, but also dead tissue whether or not it gives off CO_2 without crushing, will never produce more CO_2 when mechanically smashed. In other words the phenomenon of production of more CO_2 by crushing is characteristic only of a living tissue. By killing the seeds, and repeating the experiment, such a conclusion can easily be confirmed.

With these facts, I am proposing a new sign of life, namely, a chemical sign of irritability. It is a measurement of CO_2 due to stimulation. It is not exhalation of CO_2 which is character-

[1] The detail of this apparatus is described in *Am. J. of Physiol.*, XXXII., p. 141, 1913.

istic of life, for there are many dead matters which give off CO_2 continuously, but it is the increase of CO_2 production when crushed which is certainly a phenomenon associated with excitable tissue only. Hence, when a tissue gives off more CO_2 when mechanically crushed, or injured, it is a sure sign that this tissue is living.